IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	: 10/659,423	Confirmation No.: 4629
Applicant	: Tammy Burd-Mehta	
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TC/A.U.	: 1637	
Examiner	: Christopher M. Babic	
Docket No.	: 100/03021	
Customer No.	: 021569	
Title	: PCR Compatible Nucleic Acid Sieving Medium	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPELLANTS' BRIEF UNDER 37 CFR §1.191

Sir:

This paper is Appellants' brief filed under 37 CFR §1.191 in response to the Examiner's Final Action mailed May 14, 2008.

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I. REAL PARTY IN INTEREST

The real party in interest is Caliper Life Sciences, Inc. (formerly Caliper Technologies Corp.), which is the assignee of the entire right, title, and interest in the application involved in the appeal. The present application is a divisional of U.S. Patent Application No. 09/792,297, filed February 23, 2001, which claims the benefit of U.S. Provisional Patent Application No. 60/190,773, filed March 20, 2000. Assignment of U.S. Patent Application No. 09/792,297 by the inventor, Tammy Burd-Mehta, to Caliper Technologies Corp. is recorded on Reel/Frame 012101/0832 in the Assignment Division of the United States Patent and Trademark Office. Caliper Life Sciences, Inc., also derives its right through employment of the inventor.

II. RELATED APPEALS AND INTERFERENCES

Appellant is not aware of any related appeals, interferences, or judicial proceedings.

III. STATUS OF CLAIMS

Claims 1 and 3–10 are pending in the application. Claims 1 and 3–10 have been rejected. The rejection of each of claims 1 and 3–10 is at issue in this appeal. The limitation of claim 2 was incorporated into claim 1, and claim 2 was then canceled.

IV. STATUS OF AMENDMENTS

No amendments have been filed subsequent to the final rejection.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed subject matter is drawn to methods of performing PCR (polymerase chain reaction) and separating one or more PCR products in a medium compatible with both PCR and nucleic acid separations.

Claim 1 recites a method of performing PCR and separating one or more PCR products. The method involves mixing one or more PCR reaction components with an unpolymerized sieving medium in a channel of a microfluidic device to provide an unpolymerized PCR sieving medium within the channel. The unpolymerized PCR sieving medium is thermocycled to produce one or more PCR products. After thermocycling is completed, the sieving medium is polymerized. The one or more PCR products are then separated by flowing them through the polymerized sieving medium, which has a polymer concentration less than 0.4%.

Paragraph 0051 on page 11, paragraph 0056 beginning on page 12, and paragraph 0067 on page 15 describe mixing PCR reaction components with an unpolymerized sieving medium. These paragraphs list possible PCR reaction components as, for example, PCR extension primers, nucleotide triphosphates, thermostable enzymes, ions and buffer components such as Mg^{++}, and template DNAs. These paragraphs also support a polymer concentration that is less than 0.4%.

Thermocycling the sieving medium to produce one or more PCR products is described, for example, in paragraphs 0051 through 0054 on pages 11 and 12. Polymerizing the sieving medium following thermocycling is described in paragraph 0057 on page 13. Various methods for polymerizing a sieving medium are provided in paragraphs 00032 through 0041 on pages 7–9.

Separating the PCR product(s) by flowing the product(s) through the same sieving medium in which thermocycling was performed is described in, for example, paragraphs 0050 and 0051 on page 11 and in paragraphs 0055 through 0057 on pages 12 and 13.

Claim 1 recites a sieving medium polymer concentration less than 0.4%. Sieving mediums according to claim 1 have lower concentrations of polymer than are normally used to achieve DNA separation. A typical sieving polymer concentration used in DNA separations is about 3%. The lower concentration recited in claim 1 does not inhibit PCR and still provides

separation of polynucleotides. A low concentration polymer was initially used by the inventor as an agent to eliminate bulk flow in a channel during electrophoretic separation by acting as a dynamic coating for the channel walls; however, surprisingly, the material also provided nucleic acid separation. *See* paragraphs 0018 through 0020 on pages 3 and 4.

The polymer concentration may be about 0.35% or less (as recited in ***claim 3*** and described in lines 6 and 7 of paragraph 0019 beginning on page 3).

The polymer may comprise acrylamide (as recited in ***claim 4***) or linear acrylamide, polyacrylamide, polydimethylacrylamide, or polydimethylacrylamide/coacrylic acid (as recited in ***claim 5***), or polyethylene oxide (as recited in ***claim 6***). These polymer options are presented in paragraph 0025 on page 5 of Appellant's specification.

The one or more PCR reaction components that are mixed with the unpolymerized sieving medium may comprise one or more of a thermostable DNA polymerase, a plurality of nucleotides, a nucleic acid template, a primer which hybridizes to the nucleic acid template, or Mg^{++} (as recited in ***claim 7*** and presented in paragraph 0024 beginning on page 4 and paragraph 0051 on page 11).

The channel in which the PCR reaction component(s) and the unpolymerized sieving medium are mixed may be a microfluidic channel (as recited in ***claim 8***), and the PCR product(s) may be separated by flowing the product(s) through the sieving medium in the microfluidic channel (as recited in ***claim 9***). These steps are presented in paragraphs 0050, 0056, 0062, and 0066 on pages 11, 12, 14, and 15, respectively. The separation may be an electrophoretic separation (as recited in ***claim 10*** and presented in paragraph 0043 on page 9).

Figure 1 illustrates a microfluidic device that may be used for performing PCR and nucleic acid separations in the same medium. Channel **103** is described in paragraph 0046 on page 10 of Appellant's specification as a polymer filled channel. Using device **100** of Figure 1 to perform PCR and nucleic acid separations is described in paragraphs 0081 and 0082 on page 20.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Appellant respectfully requests that the following grounds of rejection be reviewed on appeal:

1. Claims 1, 3–5, and 7–10 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Lipshutz et al. (US 5,856,174) in view of Chetverin et al. (US 5,616,478) and further in view of Weissman et al. (US 6,395,887).

2. Claim 6 was rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Lipshutz et al. (US 5,856,174) in view of Chetverin et al. (US 5,616,478) and Weissman et al. (US 6,395,887) and further in view of Dubrow (US 5,164,055).

VII. ARGUMENT

To warrant rejection under 35 U.S.C. § 103(a), all the claim limitations must be taught or suggested by the prior art. *See* MPEP § 2142.

1. Claims 1, 3–5, and 7–10 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Lipshutz et al. (US 5,856,174) in view of Chetverin et al. (US 5,616,478) and further in view of Weissman et al. (US 6,395,887). The combination of Lipshutz et al., Chetverin et al., and Weissman et al. does not teach or suggest all of the limitations of claim 1.

Appellant respectfully submits that ***Lipshutz et al.*** do not teach any of the steps of Appellant's method claim 1, nor do they teach a device or system configured to carry out Appellant's claimed method.

In step (i) of claim 1, Appellant recites "mixing one or more PCR reaction components with an unpolymerized sieving medium in a channel of a microfluidic device to provide an unpolymerized PCR sieving medium within the channel." Lipshutz et al. do not teach mixing PCR reaction components with a sieving medium. The only mention of a sieving medium by Lipshutz et al. is the statement in column 12, lines 31–34, that capillaries used in capillary electrophoresis methods are typically filled with an appropriate separation/sieving matrix. Lipshutz et al. are silent with regard to mixing PCR reaction components with such a matrix.

Further, in the devices of Lipshutz et al., the various preparative (e.g., amplification) and analytical (e.g., microcapillary electrophoresis) reactions are carried out in "**discrete** reaction, storage and/or analytical chambers disposed within a single unit or body." *See* column 13, lines 48–53, emphasis added. The Examiner states in paragraph 3 on page 3 of the final Office action mailed May 14, 2008, "Lipshutz teaches methods of performing amplification … and capillary electrophoresis … within microfluidic devices" and "Lipshutz expressly teaches that PCR and electrophoretic separation can occur in succession within the microfluidic device," citing column 4, lines 20–45. The important point to note is that these

steps occur, as the Examiner states, in succession and, as described by Lipshutz et al., in discrete chambers

This point is illustrated by Figures 3 and 5A of Lipshutz et al., which are described as optionally including a microcapillary electrophoresis device (illustrated in Figures 4A–C). In Figure 3, chamber **210** is the chamber "in which amplification of the nucleic acids extracted from the sample is carried out." Column 18, lines 39–41. Chamber **218** is the "analytical chamber" that "optionally, or additionally comprise[s] a microcapillary electrophoresis device." Column 19, lines 21–31. One of ordinary skill in the art would appreciate that the PCR reaction components of Lipshutz et al. would be present in chamber **210**, not mixed with a sieving medium in chamber **218**. Further, chambers **210** and **218** are separated by chamber **214** and channels **212** and **216**. Chamber **214** is described in column 19, lines 17–19, as being used to "carry out additional preparative operations, such as labeling or fragmentation." Channels **212** and **216** are described simply as "fluid channels" (column 18, line 4). Therefore, one of ordinary skill in the art would appreciate that there would be no opportunity for the PCR reaction components present in amplification chamber **210** to mix with a sieving medium present in analytical chamber **218**.

In Figure 5A, chamber **514** optionally includes a microcapillary electrophoresis device. *See* column 21, lines 14–18. Chamber **514** is clearly isolated from the other chambers of the device; i.e., it is a discrete chamber in which analysis is carried out. Lipshutz et al. do not teach that thermocycling may be carried out in this chamber, nor would one of ordinary skill in the art be motivated by the figure or the description to carry out thermocycling in this chamber.

The Examiner states in the third paragraph on page 3 of the final Office action mailed May 14, 2008, "figure 1 of the specification provides a device that can be considered to contain a 'channel' comprising a chamber 107 followed by a thin fluid passage 103. It is submitted that the device embodiments taught by Lipshutz encompass such a 'channel' limitation." Even if Appellant were to agree, which Appellant does not, that the wells and chambers and channels on a device can be variously combined and termed a "channel," Lipshutz et al. do not teach Appellants limitation of "mixing one or more PCR reaction components with an unpolymerized sieving medium in a channel of a microfluidic device." As demonstrated above, reaction components of Lipschutz et al. are maintained separate from the analysis portion of the device, i.e., "in discrete chambers." Therefore, even though the Examiner submits in the

third paragraph on page 3 of the final Office action mailed May 14, 2008, "Lipshutz envisions performing an integrated PCR and capillary electrophoresis in a single 'area', 'channel', or 'chamber' within a microfluidic device," Lipshutz et al. neither envision nor teach performing PCR and electrophoresis in the same medium, i.e., in a sieving medium mixed with one or more PCR reaction components.

In step (ii) of claim 1, Appellant recites "thermocycling the PCR sieving medium to produce one or more PCR products." The Examiner has conceded in the first paragraph on page 4 of the final Office action mailed May 14, 2008, that "Lipshutz does not expressly teach performing amplification reactions within an unpolymerized medium." Further, because there is no sieving medium in any of the amplification chambers taught by Lipshutz et al., the reference cannot teach thermocycling a PCR sieving medium in situ in the amplification chamber.

In step (iii) of claim 1, Appellant recites "polymerizing the sieving medium after thermocycling is completed." Lipshutz et al. are silent with regard to any steps involved in preparing a sieving matrix, including polymerizing a sieving medium to produce a sieving matrix.

In step (iv) of claim 1, Appellant recites "separating the one or more PCR products by flowing the one or more PCR products through the polymerized sieving medium." The Examiner has conceded in the first paragraph on page 4 of the final Office action mailed May 14, 2008, that "Lipshutz does not expressly teach … separation of the amplification products by flowing the product through the polymerized form of the sieving medium." Further, as demonstrated above, Lipshutz et al. do not teach a sieving medium that corresponds to the sieving medium claimed by Appellant.

The **Chetverin et al.** reference does not overcome the demonstrated deficiencies of Lipshutz et al. The Examiner states in the second paragraph on page 4 of the final Office action mailed May 14, 2008, "Chevretin expressly teaches the amplification of nucleic acid within a sieving medium before it is cast." Appellant respectfully disagrees. Chetverin et al. do not provide any teaching regarding a sieving medium.

As described in column 4, lines 43–45, Chetverin et al. state that "nucleic acid molecules can be, like microorganisms, grown as colonies in an immobilized medium." The method is similar to the time-honored laboratory technique of growing clumps of microbes on a medium in a Petri dish and identifying the microbes based on characteristics of the individual

clumps. Chetverin et al. emphasize throughout the patent that the value of their method lies in the fact that each colony resides within a limited zone, with each colony comprising individual clones. "[D]ifferent colonies occupy separate zones within the immobilized medium, and this allows the respective clones to be observed and handled separately." *See* column 5, lines 2–5. The point of using an immobilized medium is to "retard the spreading of the colonies caused by diffusion and thus increase the resolving power of the method, especially when amplifying small nucleic acids." *See* column 5, lines 33–35. Thus, the method involves identifying amplification products by their locations within a solid medium, eliminating any need for electrophoresis or other analysis methods. One of ordinary skill in the art would recognize that the "immobilized medium" taught by Chetverin et al. is not a sieving medium and that flowing the PCR products through the "immobilized medium" would defeat the purpose of the invention. Therefore, Chetverin et al. do not teach Appellant's steps (i) through (iii) and specifically teach away from Appellant's step (iv).

The Examiner cites column 12, lines 30–40, as teaching "that sieving mediums may be impregnated with amplification enzymes before casting to prevent problems due [to] harsh polymerization conditions." Appellant respectfully submits that the Examiner has misinterpreted the text. The text states in column 12, lines 34–36, "Impregnation of pre-cast gels with enzymes and/or substrates can be a choice when the conditions of gel preparation are too strong for these labile biological substances." Appellant submits that Chetverin et al. are suggesting first casting the gels and impregnating the cast gels with components sensitive to polymerization conditions. Chetverin et al. state in column 12, lines 60–62, "Similarly, polyacrylamide or polyacrylamide: agarose gels can also be pre-cast, washed, and then impregnated with enzyme(s) and or substrates." Clearly, a "pre-cast" gel could not be washed if it were unpolymerized. Further, Appellant's interpretation of the term "pre-cast" is supported by column 12, lines 55–62, in which Chetverin et al. suggest pre-forming (i.e., pre-casting) an agarose gel layer and then soaking it in a solution containing enzymes and/or substrates.

The Examiner also cites column 19, lines 30–40, for teaching "amplification of nucleic acid within a sieving medium before it is cast; i.e., polymerized into a solidified substance." In column 19, lines 28–37, Chetverin et al. suggest the use of an uncast medium for converting RNA into cDNA. While Chetverin et al. provide no details regarding this step, cDNA synthesis is typically a sample preparation step in which DNA is synthesized from RNA

in a reaction catalyzed by a reverse transcriptase. Chetverin et al. are silent with regard to amplification of the resulting cDNA, but Appellant concludes that amplification is carried out in the cast gel to allow differentiation of the amplification products. Appellant draws this conclusion because Chetverin et al. have previously (e.g., in column 8, lines 48–51) specifically taught away from conducting amplification in an uncast medium. "It is preferred to suppress the reaction until the medium gets immobilized, especially in the case of an amplification reaction; otherwise the reaction products will be prematurely synthesized and spread throughout the medium."

The **Weissman et al.** reference does not overcome the above deficiencies of Lipshutz et al. combined with Chetverin et al. The Examiner states in the second paragraph on page 5 of the final Office action mailed May 14, 2008, that Weissman et al. teach electrophoresis using a polymer concentration that is less than 0.4%. The Examiner cites column 2 but presumably intended to cite column 9, lines 44–48, which state, "[I]n capillary electrophoresis, a neutral coated capillary … is filled with a linear polyacrylamide (e.g., 0.2% polyacrylamide)." Appellant submits that the 0.2% polyacrylamide concentration was used to provide the neutral coating and was not used as a sieving matrix. As Appellant has stated in lines 4 and 5 on page 4 of the specification, an 0.2% concentration is well below the usual threshold of sieving, and Appellant initially used a low polymer concentration "to eliminate bulk movement of the fluid by acting as a dynamic coating for the channel walls." Weissman et al. themselves used a 6% polyacrylamide gel for their electrophoresis step. *See* column 15, lines 17 and 18. Therefore, Appellant submits that Weissman et al. do not teach using a 0.2% polyacrylamide concentration in a sieving matrix. In any case, Weissman et al. do not teach the limitations demonstrated above to be absent from Lipschutz et al. combined with Chetverin et al.

As demonstrated above, the combination of Lipshutz et al., Chetverin et al., and Weissman et al. does not teach or suggest all of the limitations of Appellant's claim 1. Therefore, claim 1 is nonobvious over this combination.

Claims 3–5 and 7–10 depend either directly or indirectly from claim 1. Any claim depending from a nonobvious claim is also nonobvious. *See* MPEP § 2143.03 and *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988). Therefore, dependent claims 3–5 and 7–10 are nonobvious.

2. Claim 6 was rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Lipshutz et al. (US 5,856,174) in view of Chetverin et al. (US 5,616,478) and Weissman et al. (US 6,395,887) and further in view of Dubrow (US 5,164,055). The rejection is respectfully traversed.

As demonstrated above, Appellant's currently amended claim 1 is nonobvious over the combination of Lipshutz et al., Chetverin et al., and Weissman et al. The Examiner does not argue that Dubrow overcomes the above deficiencies of Lipshutz et al., Chetverin et al., and Weissman et al, nor does the reference do so. Therefore, claim 1 is nonobvious over the combination of Lipshutz et al., Chetverin et al., Weissman et al. and Dubrow. Claim 6 depends directly from claim 1. As any claim depending from a nonobvious claim is also nonobvious, dependent claim 6 is nonobvious.

VIII. CONCLUSION

For the reasons stated above, Appellant respectfully submits that the claims are allowable over the variously combined references. Appellant requests that the present rejections of the claims under 35 U.S.C. § 103(a) be reversed and that the application be remanded to the Examiner so that the appealed claims can proceed to allowance.

Respectfully submitted,

/Ann C. Petersen/

Ann C. Petersen
Reg. No. 55,536

CARDINAL LAW GROUP
1603 Orrington Avenue, Suite 2000
Evanston, IL 60201
Direct: 408-223-2718
Fax: 847-905-7113
ann.petersen@cardinallawgroup.com

IX. APPENDIX OF CLAIMS ON APPEAL

1. A method of performing PCR and separating one or more PCR products, the method comprising:

(i) mixing one or more PCR reaction components with an unpolymerized sieving medium in a channel of a microfluidic device to provide an unpolymerized PCR sieving medium within the channel;

(ii) thermocycling the PCR sieving medium to produce one or more PCR products;

(iii) polymerizing the sieving medium after thermocycling is completed, wherein the polymerized sieving medium has a polymer concentration that is less than 0.4%; and

(iv) separating the one or more PCR products by flowing the one or more PCR products through the polymerized sieving medium.

2. (canceled)

3. The method of claim **1**, wherein the polymer concentration of the polymerized sieving medium is about 0.35% or less.

4. The method of claim **1**, wherein the polymer comprises acrylamide.

5. The method of claim **4**, wherein the polymer comprises linear acrylamide, polyacrylamide, polydimethylacrylamide, or polydimethylacrylamide/coacrylic acid.

6. The method of claim **1**, wherein the polymer comprises polyethylene oxide.

7. The method of claim **1**, wherein the one or more PCR reaction components comprise one or more of: a thermostable DNA polymerase, a plurality of nucleotides, a nucleic acid template, a primer which hybridizes to the nucleic acid template, or Mg^{++}.

8. The method of claim **1**, comprising mixing the PCR reaction components with the sieving medium in a microfluidic channel.

9. The method of claim **8**, further comprising separating the one or more PCR products by flowing the one or more PCR products through the sieving medium in the microfluidic channel.

10. The method of claim **1**, wherein separating comprises electrophoretically separating.

X. EVIDENCE APPENDIX

No documents are attached to this Evidence Appendix.

XI. RELATED PROCEEDINGS APPENDIX

As noted on page 2 of this Brief, Appellants are not aware of any related appeals, interferences, or judicial proceedings. Inasmuch as no decisions have been rendered by a court or the Board in any related case, no documents are attached to this Related Proceedings Appendix.